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(54) Title: USE OF A WEAKLY ACID CATION EXCHANGE RESIN FOR CHROMATOGRAPHIC SEPARATION OF CARBOHYDRATES

(57) Abstract: The invention relates to the use of a weakly acid cation exchange resin for chromatographic separation of carbohydrates. In the invention the hydrophilic/hydrophobic interaction of carbohydrates, sugars and sugar alcohols with the weakly acid cation exchange resin is utilised. The weakly acid cation exchange resin is used for separation of hydrophobic saccharides, such as deoxy, methyl and anhydrosugars and anhydrosugaralcohols from more hydrophilic saccharides.

USE OF A WEAKLY ACID CATION EXCHANGE RESIN FOR CHROMATOGRAPHIC SEPARATION OF CARBOHYDRATES

FIELD OF THE INVENTION

The present invention relates to the use of a weakly acid cation exchange resin for chromatographic separation of carbohydrates. Particularly the present invention relates to utilisation of hydrophilic/hydrophobic interaction of carbohydrates, sugars and sugar alcohols with the weakly acid cation exchange resin. More particularly, the invention relates to the use of a weakly acid cation exchange resin for separation of hydrophobic saccharides, such as deoxy, methyl and anhydrosugars and anhydrosugar alcohols from more hydrophilic saccharides.

BACKGROUND OF THE INVENTION

US Patent 2 684 331 discloses a method for chromatographic separation from one another two or more substances having widely different ionization constants and at least one of the substances undergoes conciderable ionization in a dilute aqueous solution thereof. However, the method has not been used for separating carbohydrates. The examples of the US Patent 2 684 331 describe separation of salts from organic solvents, e.g. sodium chloride from formaldehyde. The method comprises an ion exchange resin having an ion identical with an ion of highly ionized solute. The ion exchange resin is either a cation exchange resin or an anion exchange resin. The cation exchange resin contains sulphonate groups as functional groups. The anion exchange resin contains quaternary ammonium groups as functional groups.

US Patent 2 911 362 describes a method comprising a chromatographic separation process employing ion exchange resins for separating two or more water soluble organic compounds from one another in an aqueous medium in the absence of an ion exchange reaction, i.e. in the substantial absence of a chemical reaction involving an absorption of ions from the aqueous medium by the resin or the introduction of ions into the solution from the resin. According to said method the ion exchange resin can be either a cation exchange resin or an anion exchange resin. The cation exchange resin may contain either sulfonate groups as functional groups or carboxylic acid groups as functional groups. The anion exchange resin contains quaternary ammonium groups as the functional groups therein. However, the separation has not been used for separation of carbohydrates.

Chromatographic separation has been used for recovery of xylose from hydrolysates of natural materials such as birch wood, corn cobs and cotton seed hulls in a method described in U.S. Patent No. 4 075 406. The resin employed in the chromatographic separation is a strongly acid cation exchanger, i.e. sulfonated polystyrene cross-linked with divinyl benzene. The use of a strongly acid cation exchanger for separation of monosaccharides e.g. xylose from magnesium sulfite cook liquor is also known from the publication WO 97/49658. The chromatographic separation has been carried out using a simulated moving bed. However, the separation of certain monosaccharides by using strong acid cation exchange resins has turned out to be difficult. According to Samuelson (Samuelson, O., Chromatography on ion-exchange resins, J. Methods Carbohyd. Chem. 6 (1972) 65 - 75), for instance, the separation of rhamnose from other carbohydrates with strong cation exchange resins has been possible by using solvents e.g. alcohol as an eluent. Rhamnose is eluted before most other carbohydrates because it has a shorter retention time than aldoses and ketoses when aqueous ethanol is used as eluent. Water would be a preferred eluent, but when it is used the problem is that the various carbohydrates, such as rhamnose, arabinose and/or xylose have the tendency to elute at almost similar retention time whereby the fractions will overlap. The separation has not been proposed to be done by water eluent.

The separation of carbohydrates, especially xylose by strong acid cation exchangers has been practised industrially but is complicated and succeeded only in one way. The method presented in US Patent No. 5 998 607 has been used especially for separating xylose from the magnesium spent liquor. The problem has been the unsufficient separation of xylose and xylonic acid. The use of a weakly acid cation exchange resin did not give any benefit when solving the problem. In the method the separation requires two steps. In the first step the cation exchanger resin is used preferably in alkaline earth form, more preferably in Mg^{2+} form and in the second step cation exchange resin is preferably in alkali-metal form (e.g. sodium). However, the separation of monosaccharides has also been found to be unsatisfactory since all the other monosaccharides elute at almost same retention time with xylose. The pH in the process was low. The resin in a divalent form seemed to separate the xylose more effectively than the resin in a monovalent form.

Publication PCT/FI00/00350 discloses sulphonated polymer resins, especially ion-exchange resins and the preparation of such resins. The poly-

mer is a styrenedivinylbenzene copolymer, strongly acid cation exchange resin. The cross-linking agent can also be isoprene, allyl methylacrylate, vinyl methacrylate, glycol methacrylate or glycol diacrylate. According to the publication PCT/FI00/00350 the sulphonated polymer resin can be used as a chromatographic resin, ion exchange resin or as a catalyst resin.

US Patent 4 359 430 describes a process for recovering betaine from natural sources such as beet molasses, residue molasses and vinasses. The process uses a chromatographic column of strong acid cation exchange resin in alkali metal form, sodium being generally the preferred alkali metal. Water is used as eluent in the process. The process results in three fractions. The first fraction is a non sugar waste fraction, the second is a sugar containing fraction and the third fraction consists substantially of betaine.

Publication WO 96/10650 discloses a method for processing a beet derived sucrose containing solution to yield a sucrose enriched fraction and a fraction enriched with a second organic compound, especially such as betaine, inositol, raffinose, galactinol or serine and other amino acids. A strong acid cation exchanger preferably in sodium or potassium form is used for the separation of the fractions. From Finnish Patent No. 960 225 it is also known a method for fractioning of molasses by using a strong acid cation exchanger.

Anion exchange resins have been used for separating fructose from glucose. Y. Takasaki (Agr. Biol. Chem. 36 (1972) pages 2575 - 77) and B. Lindberg et al. (Carbohyd. Res. 5 (1967), pages 286 - 291) describe the use of an anion exchanger in bisulfite form for the separation of sugars. Water is used as eluent. However, the use of anion exchange resins does not result in a good xylose separation because the xylose is overlapping by other sugars. The separation of rhamnose has not been suggested. The separation of fructose and glucose by an anion exchanger in a bisulfite or sulfite form is known also from Patent FR-2 117 558.

U.S. Patent No. 5 084 104 discloses a method for separation of xylose from pentose-rich solution, e.g. from birch wood. A chromatographic column which comprises a strong base anion exchange resin is used. The anion exchange resin is in sulfate form. Using this method xylose is retarded most strongly, but the other monosaccharides are eluted faster.

A method for preparing of L-arabinose is known from the publication WO 99/57326 where the process is charachterized by contacting plant fibers with an acid to hydrolyze the fibers under such conditions that the L-arabinose

ingredients contained in the plant fibers are selectively obtained. U.S. Patent No. 4 880 919 discloses a process for separating arabinose from mixtures of monosaccharides containing arabinose and other aldopentoses and aldohexoses by adsorption on sulfonated polystyrene divinyl benzene cross-linked ion exchange resins in with Ca^{2+} and NH_4^+ forms and desorpting the adsorbate with water. A process for production of crystalline L-arabinose is known from U.S. Patent No. 4 816 078.

The preparation of arabinose is also known from the US Patent 4 664 718. In the method the arabinose is separated from the monosaccharide mixture containing also other aldopentoses and aldohexoses. The feed is contacted with calcium-Y-type or calcium-X-type zeolite and arabinose is adsorbed selectively. The desorption is conducted with water or ethanol.

Publication DE 3 545 107 describes a method for preparation of rhamnose from arabic gum. A strongly acid cation exchange resin is used for the separation of the sugar and rhamnose is purified by adsorption with an activated charcoal. Arabinose is also separated with this method.

Barker, S.A. et al (Carbohydrate Research, 26 (1973) 55 - 64) have described the use of poly(4-vinylbenzeneboronic acid) resins in the fractionation and interconversion of carbohydrates. In the method water is used as an eluent. The best yield of fructose was received when the pH was high. The resins have been used to displace the pseudo equilibrium established in aqueous alkali between D-glucose, D-fructose and D-mannose to yield D-fructose.

Surprisingly it has been found out that when using weakly acid cation exchange resins an improved chromatographic separation of carbohydrates is accessed. In addition to other features the order of separation seems to be affected by the hydrophobic/hydrophilic interactions of carbohydrates with resin and an improved separation of carbohydrates is resulted. Other commonly known features in chromatographic separation of carbohydrates on ion exchange resins include e.g. ion exclusion and size exclusion. If the resin is in the hydrophilic form the most hydrophobic monosaccharides seem to elute first and the most hydrophilic last. This results in a different elution order than previously found.

SUMMARY OF THE INVENTION

The above mentioned objects and others are accomplished by the present invention in which a weakly acid cation exchange resin is used to

separate monosaccharides, disaccharides or oligosaccharides chromatographically. Preferably the ion exchange resin used is a acrylic weakly acid cation exchanger with a carboxylic functional group cross-linked with from about 1 to about 20 %, preferably from about 3 to about 8 % divinyl benzene. The resin is in H^+, K^+, Na^+, Mg^{2+} or Ca^{2+} form and also other ion forms can be used. This kind of resin proved to be more efficient than the earlier tested, e.g. the polystyrene matrix resins. This seems to be also affected to the fact that aromatic based resins are more hydrophobic than the acrylic based resins.

The weakly acid cation exchange resin is used for the separation of carbohydrates, particularly hydrophobic saccharides. Preferably the weakly acid cation exchange resin is used for the separation of hydrophobic monosaccharides, such as deoxy, methyl and anhydrosugars and sugar alcohols from more hydrophilic saccharides. Most preferably the weakly acid cation exchange resin is used for separating saccharides consisting of the group of hexoses, such as ketohexoses, aldohexoses, pentoses, such as ketopentoses, aldopentoses, corresponding sugars and sugar alcohols and mixtures thereof, e.g. glucose, fructose, rhamnose, anhydrosorbitol, sorbitol, erythritol, inositol, arabinose, xylose and xylitol. Sucrose, betaine and amino acid containing solutions can also be separated advantageously. The weakly acid cation exchange resin is also used for separating anhydrosugars from corresponding sugars, separating anhydrosugar alcohols from corresponding sugar alcohols, separating sugars, sugar alcohols and their anhydro forms from salts and for separating erythritol from inositol. When the resin is in a hydrophilic form the most hydrophobic monosaccharide seems to be eluted first and the most hydrophilic monosaccharide seems to be eluted last. This seems to be affected by the hydrophilic/hydrophobic interactions of the resin and the components.

The raw materials containing aforementioned carbohydrates, hydrolysates and extracts from plants or raw materials converted thereof containing aforementioned carbohydrates for which the weakly acid cation exchanger is to be used are e.g. xylose process streams, sucrose process streams, starch or sucrose based streams, for example maltose, glucose or fructose process streams or their process side streams.

The weakly acid cation exchange resin described above is used in a chromatographic column. The resin is used in a chromatographic column at temperatures from 10 to 95 °C preferably from 40 to 95 °C, more preferably

from 60 to 95 °C. It is known that a higher separation temperature decreases viscosity and improves the separation performance of the sugars.

The eluent used in the chromatographic separation is water for instance demineralized water or condensate water or some other aqueous solution, alcohol or a mixture thereof. Preferably the eluent is water.

The order of elution of the monosaccharides in the present invention is different from the elution order obtained earlier by using strong base resins in bisulfite or sulfate form or using strong acid cation exchange resins. As one preferred example of the invention rhamnose can be separated before more hydrophilic monosaccharides. This allows the rhamnose to be recovered with good yield as a highly purified fraction. When separating betaine, erythritol and inositol the carbohydrates are separated in said order after betaine. If rhamnose is separated from other monosaccharides it is advantageous that rhamnose is eluted first. If erythritol and inositol are separated from a betaine containing solution it is advantageous that erythritol is separated before inositol.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention.

FIG. 1 is a graphical presentation of the elution profiles and pH obtained from Example 1.

FIG. 2 is a graphical presentation of the elution profiles and pH obtained from Example 2.

FIG. 3 is a graphical presentation of the elution profiles and pH obtained from Example 3.

FIG. 4 is a graphical presentation of the elution profiles and pH obtained from Example 4.

FIG. 5 is a graphical presentation of the elution profiles and pH obtained from Example 5.

FIG. 6 is a graphical presentation of the elution profiles and pH obtained from Example 6.

FIG. 7 is a graphical presentation of the elution profiles and pH obtained from Example 7.

DETAILED DESCRIPTION

A solution containing carbohydrates is subjected to a chromatographic separation. The separation is performed in a chromatographic sepa-

ration column. The chromatographic column is filled with a weakly acid cation exchange resin.

The resin used in the chromatographic column is suitably a weakly acid acrylic cation exchanger having carboxylic functional groups. The weakly acid acrylic cation exchange resin is derived from the group consisting of acrylate esters, like methyl acrylate, ethyl acrylate, butyl acrylate and methyl methacrylate or acrylonitrile or acrylic acids of mixtures thereof. The skeleton of the resin can also be other than acrylic. The active functional group can also be other than carboxylic group, e.g. it can be selected from other weak acids. The acrylic cation exchange resin is cross-linked with a compound from the group consisting of aromatic cross-linker, like divinyl benzene or with a aliphatic cross-linker like isoprene, 1,7-octadiene, trivinylcyclohexane, diethylene glycol divinylether. The degree of the cross-linkage of the resin is from about 1 to about 20 %, preferably about 3 to about 8 % divinyl benzene. The average particle size of the weakly acid cation exchange resin is from 10 to 2000 micrometers, preferably from 100 to 400 micrometers The resin can be regenerated into mainly H^+, K^+, Na^+, Mg^{2+} or Ca^{2+} form. Other ion forms may also be used.

The carbohydrate solution to be fractioned is optionally pretreated first by filtration, which can be done using a pressure filter and diatomaceous earth as filter aid. The feed solution is optionally adjusted to pH from 1 to 11, preferably from 2 to 10, more preferably from 2 to 4 and from 5 to 10 e.g. with sodium hydroxide solution. After this the solution may be optionally filtered before chromatographic separation.

Also the dry substance content of the feed solution is adjusted to an appropriate level before chromatographic separation.

A feeding device may be used for feeding the solution on the surface of the resin bed. The flow of the solution can be downwards or upwards, downwards is preferred. The temperature of the column and feed solution and eluent is 10 to 95 °C, preferably 40 to 95 °C and most preferably approximately from 60 to 95 °C. This is accomplished by preheating the solution. The eluent used is either water or solvent. Water can be for instance demineralized water or condensate water. Solvent can be an aqueous solution or alcohol or a mixture thereof. Preferably the eluent is water for efficient separation.

The feed solution is eluted in the column by feeding preheated water, for instance demineralized water or condensate water or some other

aqueous solution or alcohol or a mixture thereof into the column. The flow rate in the column is adjusted to an appropriate level.

The fractions of the outcoming solution are collected at appropriate intervals and optionally the composition of the fractions is analysed. The outcoming streams can be followed by online instruments.

The following examples illustrate the present invention. They are not to be construed to limit the claims in any manner whatsoever.

Example 1

Chromatographic separation of xylose crystallization run-off with a H^+/Mg^{2+} -form resin

Xylose crystallization run-off, which was beech wood based originally from Mg based si- cooking liquor was subjected to a chromatographic separation. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 12 GC) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate - based resin. The height of the resin bed was about 0,70 m. The cross-linkage degree of the resin was 6,0% DVB and the average particle size of the resin was 0,26 mm. The resin was regenerated into mainly H^+-form (94%) and partly Mg^{2+}-form (6%) and a feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 65°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The pH of the feed solution was 3,5.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with Dionex HPLC equipment with pulsed electrochemical detector and CarboPac PA1™ anion exchange column (water and 0,2 M NaOH as eluents).

Resin gives a good separation of rhamnose from other monosaccharides. Arabinose and rhamnose are eluted in the end of the separation profile. The pH of the effluent was between 3 to 4. The results are shown graphically in FIG. 1.

Example 2

Chromatographic separation of anhydrosorbitol (1,4-anhydro-D-glucitol) and sorbitol with a Na^+-form resin

A solution containing anhydrosorbitol (1,4-anhydro-D-glucitol) and sorbitol was subjected to a chromatographic separation. The solution was prepared by dissolving pure anhydrosorbitol and sorbitol into ion-exchanged water. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 12 GC) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate –based resin. The height of the resin was about 0,70 m. The cross-linkage degree of the resin was 6 % DVB and the average particle size of the resin was 0,26 mm. The resin was in Na^+ -form. The pH of the resin was high after the manufacturing process. A feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 65°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The feed solution composed of 50% on dry substance (DS) anhydrosorbitol and 50% on DS sorbitol.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with HPLC (Pb^{2+}-form resin, 0,6 ml/min, 85°C water eluent).

Components were eluted from the column in the following order: anhydrosorbitol and sorbitol. The elution order seems to be consistent with the hydrophobic/hydrophilic –nature of the components. The pH of the effluent was between 7,5 to 11. The resin separated components from each other well. The results are shown graphically in FIG. 2.

Example 3

Chromatographic separation of sucrose, glucose and fructose with a Na^+-form resin

A solution containing sucrose, glucose and fructose was subjected to a chromatographic separation. The solution was prepared by dissolving pure sucrose, glucose and fructose into ion-exchanged water. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 12 GC) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate –based resin. The height of the resin was about 0,70 m. The cross-linkage degree of the resin was 6 % DVB and the average particle size of the resin was 0,26 mm. The resin was in Na^+ -form. The pH of the resin was high after the manufacturing process. A feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 65°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The feed solution

composed of 33% on dry substance (DS) sucrose, 33% on DS glucose and 33% on DS fructose.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with HPLC (Na^+-form resin, 0,8 ml/min, 0,003 M Na_2SO_4, 85°C).

First sucrose eluted from the column as a separate peak. Glucose and fructose eluted together as a second peak after sucrose. Resin gives a good separation between sucrose and monosaccharides. The pH of the effluent was between 9 to 11. The results are shown graphically in FIG. 3.

Example 4

Chromatographic separation of sodium chloride, betaine, erythritol and inositol with a Na^+-form resin

A solution containing betaine, erythritol, inositol and sodium chloride (NaCl) was subjected to a chromatographic separation. The solution was prepared by dissolving pure betaine, erythritol, inositol and sodium chloride into ion-exchanged water. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 12 GC) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate –based resin. The height of the resin bed was about 0,70 m. The cross-linkage degree of the resin was 6 % DVB and the average particle size of the resin was 0,26 mm. The resin was in Na^+ -form. The pH of the resin was high after the manufacturing process. A feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 80°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The feed solution composed of 30% on dry substance (DS) betaine, 30% on DS inositol, 30% on DS erythritol and 10% on DS sodium chloride.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with HPLC (Ca^{2+}-form resin, 0,8 ml/min, 0,001 M $Ca(NO_3)_2$, 85°C).

Components were eluted from the column in the following order: sodium chloride, betaine, erythritol and inositol. The elution order of betaine and carbohydrates seems to be consistent with the hydrophobic/hydrophilic – nature of the components. The resin separated components from each other well. The pH of the effluent was between 6 to 9. The results are shown graphically in FIG. 4.

Example 5

Chromatographic separation of sodium chloride, betaine, sucrose and mannitol with a Na^+-form resin

A solution containing betaine, sucrose, mannitol and sodium chloride (NaCl) was subjected to a chromatographic separation. The solution was prepared by dissolving pure betaine, sucrose, mannitol and sodium chloride into ion-exchanged water. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex CA 12 GC) manufactured by Finex Oy, Finland. The resin was an

ethyl acrylate –based resin. The height of the resin was about 0,65 m. The cross-linkage degree of the resin was 6 % DVB and the average particle size of the resin was 0,26 mm. The resin was in Na^+ -form. The pH of the resin was high after the manufacturing process. A feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was approximately 80°C. The flow rate in the column was adjusted to 4 ml/min.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution. The feed solution composed of 30% on dry substance (DS) betaine, 30% on DS sucrose, 30% on DS mannitol and 10% on DS sodium chloride.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with HPLC (Na^+-form resin, 0,8 ml/min, 0,003 M Na_2SO_4, 85°C).

First sodium chloride was eluted from the column. Sucrose and betaine were eluted from the column together as a one peak overlapping with salts to some extent. Mannitol was eluted from the column as a separate peak after sucrose and betaine. Resin separated mannitol from sucrose and betaine well. The pH of the effluent was between 7 to 11. The results are shown graphically in FIG. 5.

Example 6

Chromatographic separation of beet molasses with weakly acid cation exchange resin

Beet molasses was subjected to a chromatographic separation. The separation was performed in a laboratory scale chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 16 GC, manufactured by Finex Oy, Finland). The resin was methyl acrylate based. The cross-linkage degree of the resin was 8 % DVB and the average particle size about 0,23 mm. The resin was in Na^+-form prior the separation.

The height of the resin was about 0,70 m. The pH of the resin was quite high after the manufacturing process (pH about 9 - 10). A feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was approximately 80 °C. The flow rate in the column was adjusted to 4 ml/min. The feed solution was filtered via filter prior the separation. The pH of the feed solution was about 8,2.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected in 3 min interval. The composition of the samples was analysed with HPLC (Na^+-form column, 0,8 ml/min, 0,003 M Na_2SO_4, 85 °C).

Salts eluted out of the column first. Sucrose and betaine are eluted at the same retention time and overlapped with the salts to some extent. Amino acids eluted mainly at the back slope of the profile. The pH of the efflu-

ent was between 7,5 to 10. The results are shown graphically in FIG. 6. Table 1 shows the amino acid concentration of samples 21 to 39.

Table 1. Amino acid concentration

Sample number	RDS g/100g	Amino acids % on DS	Amino acids g/100g
21	20,54	1,8	0,370
23	16,36	3,1	0,507
25	5,09	8,5	0,433
26	3,58	13,0	0,465
27	2,47	16,5	0,408
29	1,28	4,9	0,063

Example 7

Chromatographic separation of fructose crystallization run-off with a Na^+-form resin

Concentrated and heat treated fructose crystallization run-off was subjected to a chromatographic separation. The separation was performed in a laboratory chromatographic separation column as a batch process. The column with a diameter of 0,045 m was filled with an acrylic weakly acid cation exchange resin (Finex™ CA 12 GC) manufactured by Finex Oy, Finland. The resin was an ethyl acrylate -based resin. The height of the resin bed was about 0,70 m. The cross-linkage degree of the resin was 6 % DVB and the average particle size of the resin was 0,26 mm. The resin was regenerated into Na^+-form and a feeding device was placed at the top of the resin bed. The temperature of the column and feed solution and eluent water was 60°C. The flow rate in the column was adjusted to 4 ml/min. The pH of the feed solution was adjusted to 7 with sodium hydroxide.

The chromatographic separation was carried out as follows:

Step 1:

The dry substance of the feed solution was adjusted to 25 g dry substance in 100 g solution according to the refractive index (RI) of the solution.

Step 2:

100 ml of preheated feed solution was pumped to the top of the resin bed.

Step 3:

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4:

10 ml samples of the outcoming solution were collected at 3 min intervals. The composition of the samples was analysed with HPLC (Na^+-form resin, 85°C, water eluent, 0,8 ml/min).

Resin separates well fructose and oligosaccharides formed in thermal acid breakdown of fructose. Oligosaccharides are eluted from the column faster than fructose. The pH of the effluent was between 6 to 11. The results are shown graphically in FIG. 7.

Example 8

The use of acryl-based resins in liquid chromatography while using deionized water and an approximately 30 percent by weight water-ethanol solution as eluents

In a liquid chromatography test, acryl-based resins manufactured by Finex Oy (Finland) cross-linked with DVB (divinyl benzene) were used as the stationary phases. The cross-linking degrees of the resins were 4 % DVB (CA08GC) and 6 % DVB (CA12GC). The average particle size of the resins was 375 µm. A strong acid styrene-based sulphonated cation exchange resin (CS08G) of the same manufacturer, having an average particle size of 395 µm, was used as a comparison resin.

Pharmacia Biotech FPLC™ liquid chromatography equipment was used in the column tests, the equipment comprising a pump, a jacketed glass column, with temperature control, an RI (Refractive Index) detector and a computer used in collecting the measurement data. An *RI-98 SCOPE* refractive index detector was used in on-line analysis of the effluent. The test column was a *Pharmacia Biotech XK16* with a diameter of 1.6 cm. Resins in Na^+ form were used in the tests, and approximately 60 ml of resin (water-swollen resin) was packed in the column. Bed height in water was approximately 30 cm.

In the column tests, deionized water and an approximately 30 percent by weight water-ethanol solution, with air removed from them by vacuum suction, were used as eluents. The flow rate of the eluent was 1 ml/min in all

tests and the tests were carried out at a temperature of 25°C. The column was equalised before the measurements by pumping said eluent through it until the resin was equalised and the bottom level of the RI detector remained constant.

The samples used in the measurements were made with the eluent used in the operation. The xylose ($C_5H_{10}O_5$) and rhamnose monohydrate ($C_6H_{12}O_5$*1 H_2O) content of the sample solutions was 70 g/l and Blue Dextran 1.5 g/l was used as the unretarded component. The sample volume was 0.5 ml. The porosity of the resin bed was determined from the pulse response values of the Blue Dextran runs.

The chromatographic parameters were calculated by the moment method. Before calculating the parameters, all chromatograms were processed with the *Jandel Scientific Peak Fit .v4* program, by means of which the bottom level of the curves was corrected and the negative peak caused by ethanol was removed. The division constants and the separation factor of xylose and rhamnose were calculated from the pulse responses as follows:

$$K_i = \frac{V_i - V_{BD}}{V_S} = \frac{\left(\frac{V_i}{\upsilon_n} - \frac{z\varepsilon}{\upsilon_n / (\pi r^2)} - \frac{V_i}{2\upsilon_n}\right)}{\frac{z(1-\varepsilon)}{\upsilon_n / (\pi r^2)}} \qquad (1)$$

wherein K_i = the distribution constant of sub-type i
V_i = the retention volume of sub-type i
V_{BD} = the retention volume of the unretarded sub-type (Blue Dextran)
V_S = volume of stationary phase
υ_n = flow rate of eluent
z = height of stationary phase
ε = porosity
r = radius of column
V_I = volume of supplied sample

$$\alpha_{ij} = \frac{V_i - V_{BD}}{V_j - V_{BD}}$$

wherein α_{ij} = separation factor of sub-type i with respect to sub-type j
V_j = retention volume of sub-type j

Table 2

Resin	Eluent	z	ε	K_{rham}	K_{xyl}	$\alpha_{rham/xyl}$
		cm	-	-	-	-
CA08GC	Water	29.2	0.34	0.39	0.56	1.44
	EtOH 29.3 w-%	18.4	0.35	0.60	1.14	1.89
CA12GC	Water	30.0	0.34	0.25	0.42	1.66
	EtOH 29,4 w-%	23.3	0.34	0.53	1.03	1.95
CS08G	Water	29.9	0.37	0.47	0.53	1.13
	EtOH 29,3 w-%	26.5	0.36	0.70	0.85	1.21

The results show that adding ethanol to the eluent improves the separation of xylose and rhamnose. The results show that weakly acid cation exchange resin is better for chromatographic separation of xylose and rhamnose than a strong acid cation exchange resin.

CLAIMS

1. The use of a weakly acid cation exchange resin for chromatographic separation of carbohydrates from each other.

2. The use of a weakly acid cation exchange resin according to claim 1 characterized in that the resin is a acrylic weakly acid cation exchange resin.

3. The use of a weakly acid cation exchange resin according to claim 1 or 2 characterized in that the acrylic resin is derived from the group consisting of an acrylate ester or acrylonitrile or acrylic acids or mixtures thereof.

4. The use of a weakly acid cation exchange resin according to claim 3 characterized in that the acrylate ester is selected from the group consisting of methyl methacrylate, methyl acrylate, ethyl acrylate and butyl acrylate.

5. The use of a weakly acid cation exchange resin according to any one of claims 1 to 4 characterized in that resin is in the form of H^+, Na^+, K^+, Ca^{2+} or Mg^{2+}.

6. The use of a weakly acid cation exchange resin according to any one of claims 1 to 5 characterized in that the resin is cross-linked with an aromatic cross-linker, such as divinyl benzene (DVB) or with an aliphatic cross-linker, such as isoprene, 1,7-octadiene, trivinylcyclohexane or diethylene glycol divinylether.

7. The use of a weakly acid cation exchange resin according to claim 6 characterized in that the degree of cross-linkage of the resin is from about 1 to about 20 % by weight DVB.

8. The use of a weakly acid cation exchange resin according to claim 7 characterized in that the degree of cross-linkage of the resin is from about 3 to about 8 % by weight DVB.

9. The use of a weakly acid cation exchange resin according to any one of claims 1 to 8 characterized in that the average particle size of the resin is from 10 to 2000 micrometers.

10. The use of a weakly acid cation exchange resin according to claim 9 characterized in that the average particle size of the resin is from 100 to 400 micrometers.

11. The use of a weakly acid cation exchange resin according to any one of claims 1 to 10 characterized in that the pH of the feed solution is from 1 to 11.

12. The use of a weakly acid cation exchange resin according to claim 11 characterized in that the pH of the feed solution is from 2 to 10.

13. The use of a weakly acid cation exchange resin according to claim 11 characterized in that the pH of the feed solution is from 2 to 4 or from 5 to 10.

14. The use of a weakly acid cation exchange resin according to any one of claims 1 to 13 characterized in that the temperature of the column, the feed solution and the eluent is from 10 to 95 °C.

15. The use of a weakly acid cation exchange resin according to claim 14 characterized in that the temperature of the column, the feed solution and the eluent is from 40 to 95 °C.

16. The use of a weakly acid cation exchange resin according to any one of claim 15 characterized in that the temperature of the column, the feed solution and the eluent is from 60 to 95 °C.

17. The use of a weakly acid cation exchange resin according to any one of claims 1 to 16 characterized in that the eluent is selected from the group consisting of water, an alcohol and a mixture thereof.

18. The use of a weakly acid cation exchange resin according to claim 17 characterized in that the eluent is water.

19. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that the carbohydrates to be separated are sugars and sugar alcohols.

20. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that the hydrophilic carbohydrates are separated from the more hydrophobic carbohydrates.

21. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that anhydrosugars are separated from the corresponding sugars.

22. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that anhydrosugar alcohols are separated from the corresponding sugar alcohols.

23. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that the carbohydrates to be separated are monosaccharides.

24. The use of a weakly acid cation exchange resin according to claim 23 characterized in that the monosaccharide is L-rhamnose.

25. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that the carbohydrates to be separated are disaccharides or oligosaccharides.

26. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that carbohydrates to be separated are selected from the group consisting of hexoses, such as ketohexoses, aldohexoses, pentoses, such as ketopentoses, aldopentoses, corresponding sugars and sugar alcohols and mixtures thereof, e.g. glucose, fructose, rhamnose, anhydrosorbitol, sorbitol, erythritol, inositol, arabinose, xylose and xylitol.

27. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 wherein betaine and amino acids are separated from the carbohydrates.

28. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that the sugars, sugar alcohols and their corresponding anhydroforms are separated from salts.

29. The use of a weakly acid cation exchange resin according to any one of claims 1 to 18 characterized in that sugars, sugar alcohols and their corresponding anhydroforms are separated from ionic substances.

30. The use of a weakly acid cation exchange resin according to claim 28 or 29 characterized in that sucrose and non-sugar components are recovered in beet molasses separation.

31. The use of a weakly acid cation exchange resin according to claim 30 characterized in that when molasses is subjected to separation sugar and non-sugar components are recovered.

32. The use of a weakly acid cation exchange resin according to claim 31 characterized in that amino acids are separated with sucrose fraction.

33. The use of a weakly acid cation exchange resin according to claim 32 characterized in that mannitol is separated from betaine, sucrose and salts.

34. The use of a weakly acid cation exchange resin according to any of the claims 1 to 18 characterized in that fructose is separated from disaccharides and oligosaccharides.

35. The use of a weakly acid cation exchange resin according to any of the claims 1 to 18 characterized in that monosaccharides are separated from the oligosaccharides.

36. The use of a weakly acid cation exchange resin according to any one of the claims 1 to 18 characterized in that the monosaccharides are separated from the disaccharides.

37. The use of a weakly acid cation exchange resin according to any of the claims 1 to 18 characterized in that the sucrose is separated from fructose and glucose.

38. The use of a weakly acid cation exchange resin according to any of the claims 1 to 18 characterized in that erythritol, inositol and mannitol are separated from betaine.

39. The use of a weakly acid cation exchange resin according to claim 38 characterized in that erythritol is separated from inositol.

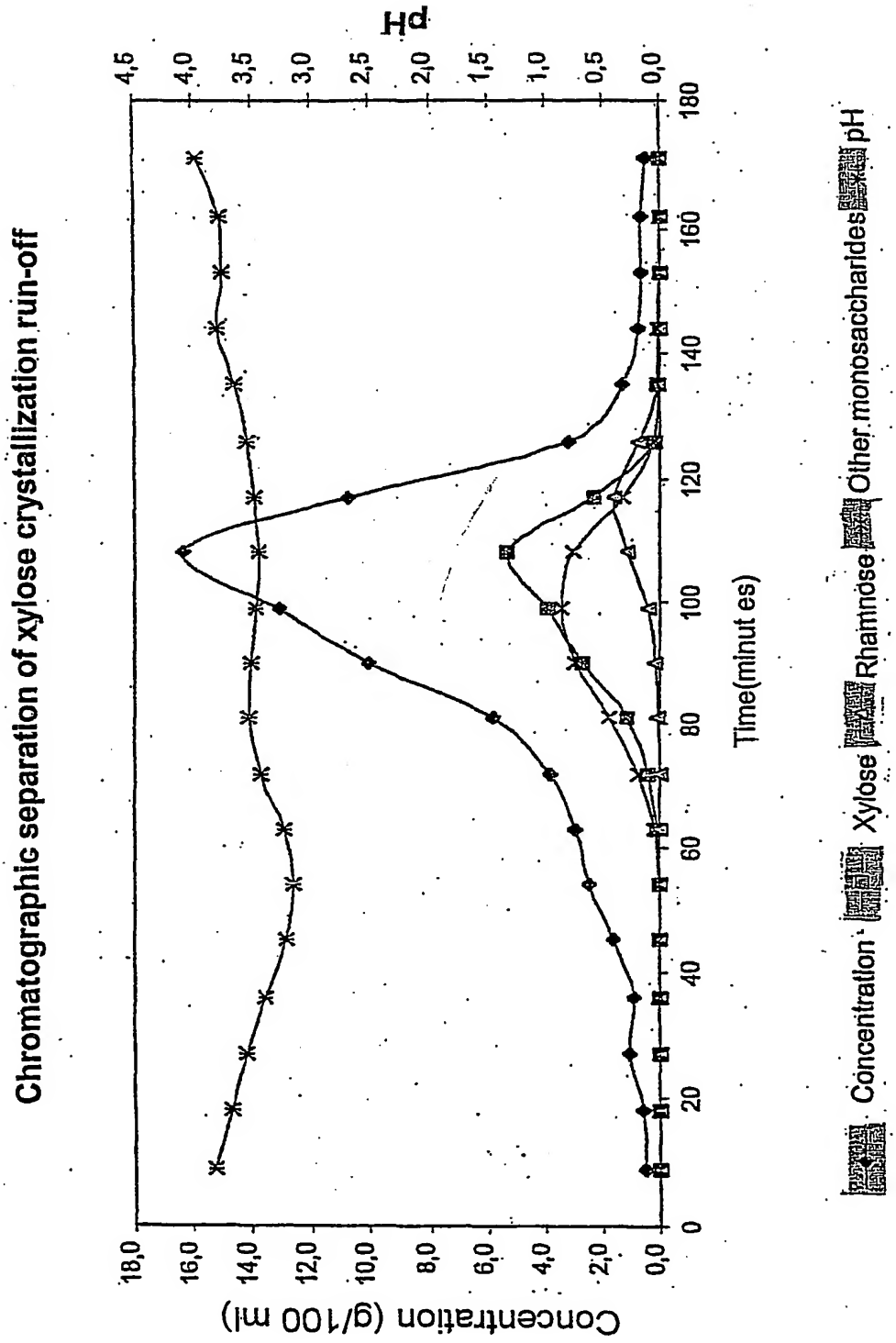
Chromatographic separation of xylose crystallization run-off
Concentration (g/100 ml)
pH
Time(minut es)
Concentration
Xylose
Rhamnose
Other monosaccharides
pH

FIG. 1

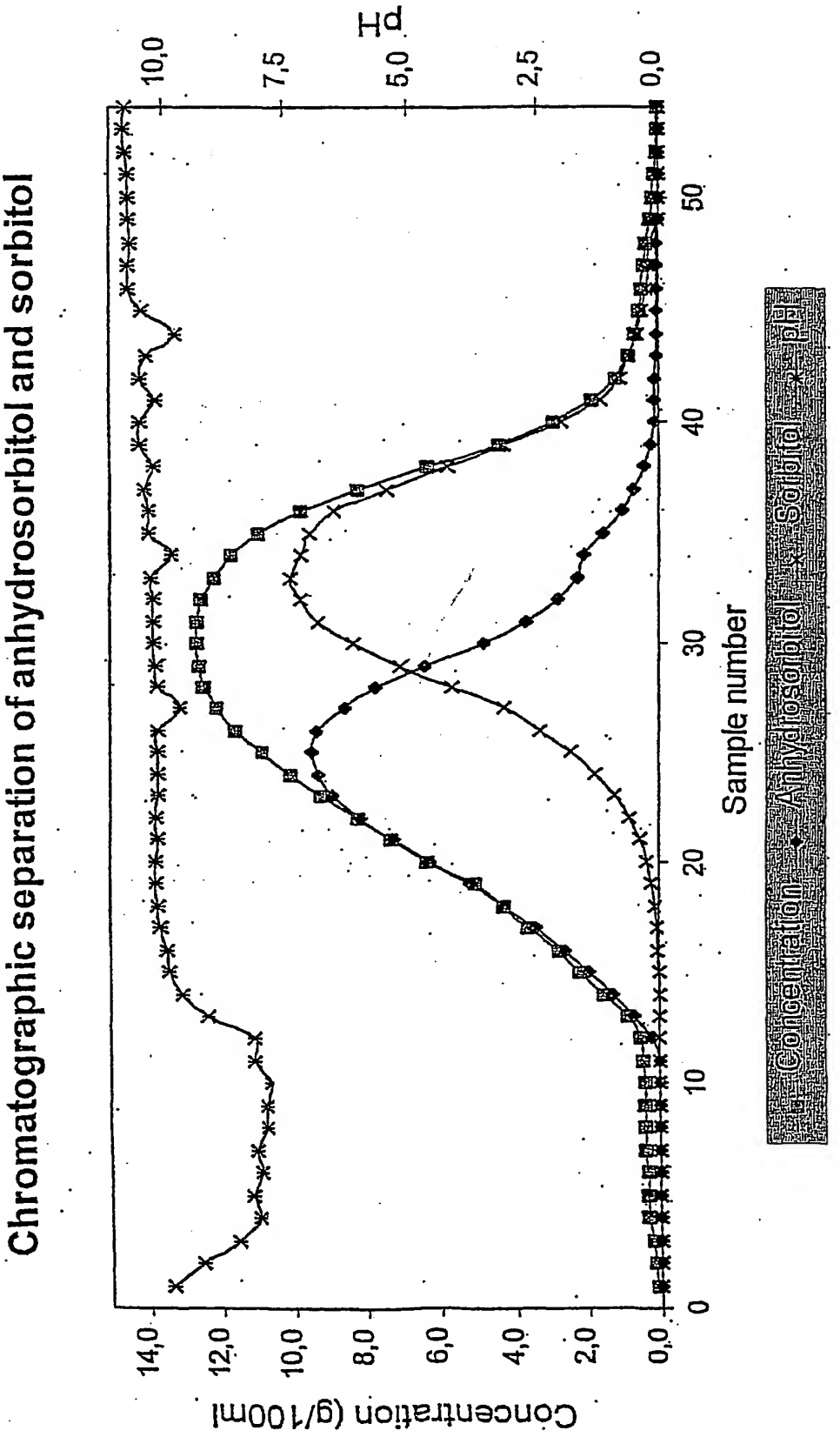
Chromatographic separation of anhydrosorbitol and sorbitol
Concentration (g/100ml
pH
Sample number
Concentration
Anhydrosorbitol
Sorbitol
pH

FIG. 2

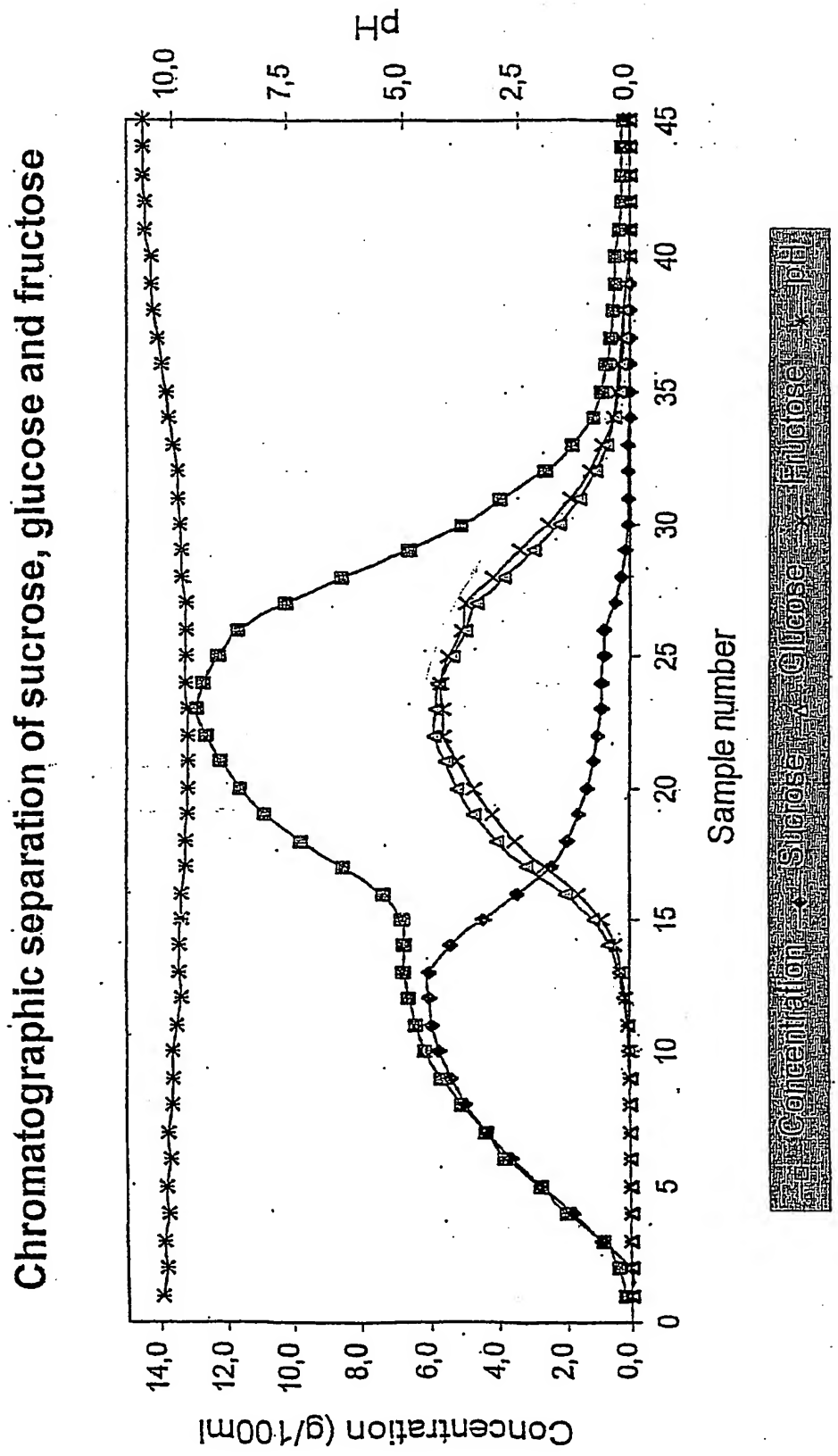
Chromatographic separation of sucrose, glucose and fructose
Concentration (g/100ml)
pH
Sample number
Concentration
Sucrose
Glucose
Fructose
pH

FIG. 3

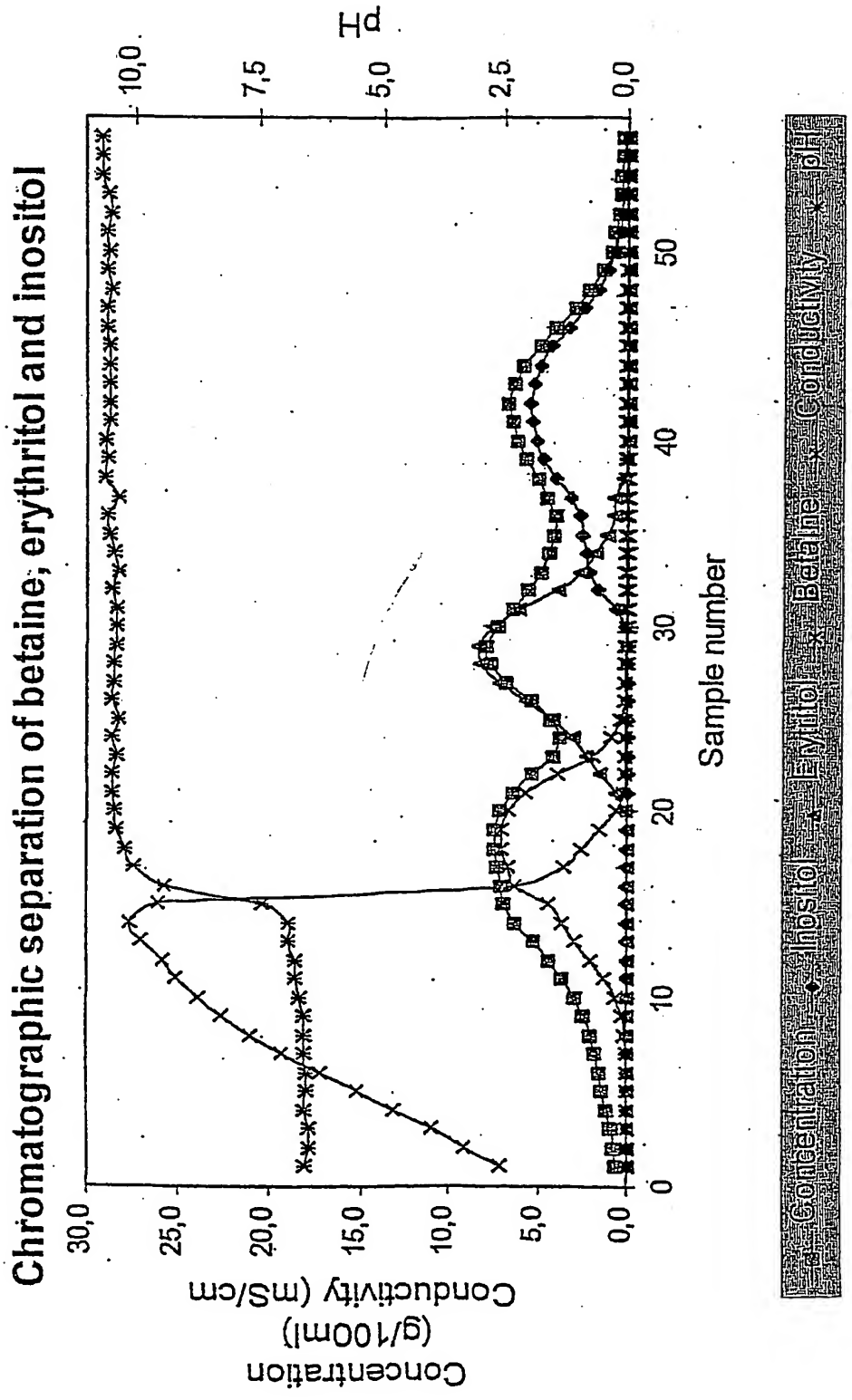
Chromatographic separation of betaine, erythritol and inositol
Concentration (g/100ml)
Conductivity (mS/cm
pH
Sample number
Concentration
Inositol
Erythritol
Betaine
Conductivity
pH
30,0
25,0
20,0
15,0
10,0
5,0
0,0
10,0
7,5
5,0
2,5
0,0
0
10
20
30
40
50

FIG. 4

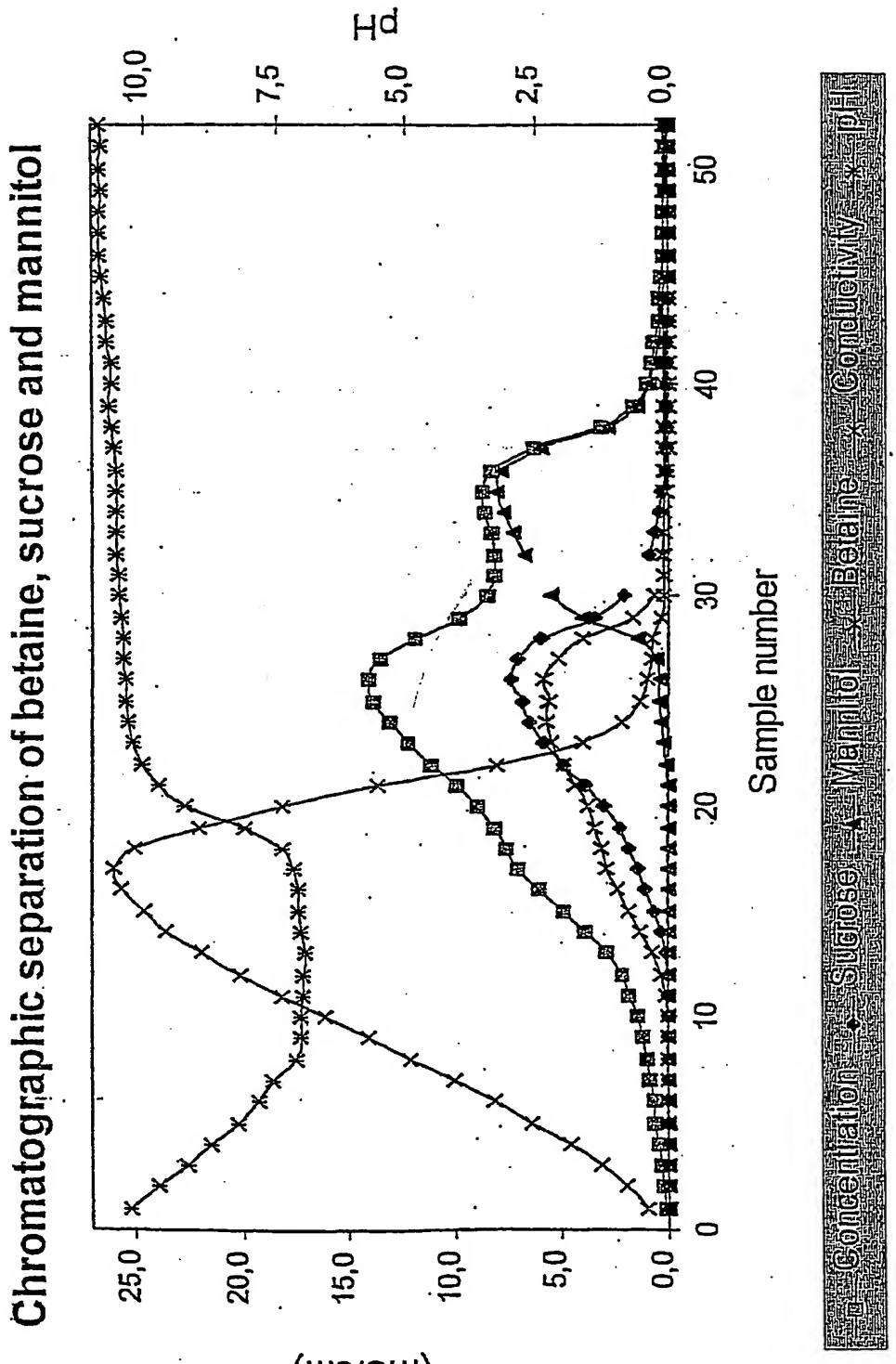
Chromatographic separation of betaine, sucrose and mannitol
Concentration (g/100ml) Conductivity (mS/cm)
pH
Sample number
Concentration
Sucrose
Mannitol
Betaine
Conductivity
pH
25,0
20,0
15,0
10,0
5,0
0,0
10,0
7,5
5,0
2,5
0,0
0
10
20
30
40
50

FIG. 5

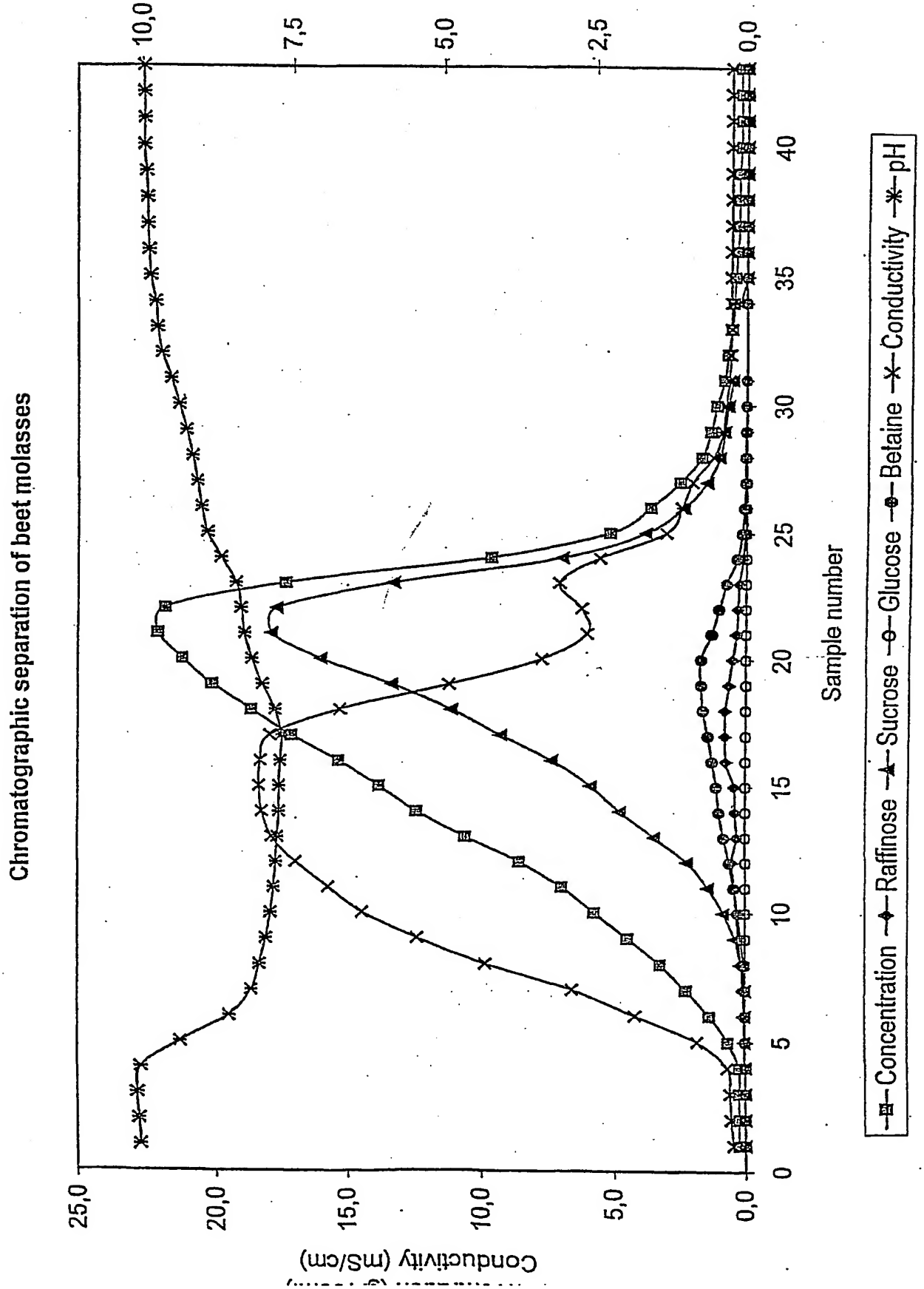
Chromatographic separation of beet molasses
Conductivity (mS/cm)
pH
Sample number
Concentration
Raffinose
Sucrose
Glucose
Betaine
Conductivity
pH
25,0
20,0
15,0
10,0
5,0
0,0
10,0
7,5
5,0
2,5
0,0
0
5
10
15
20
25
30
35
40

FIG 4

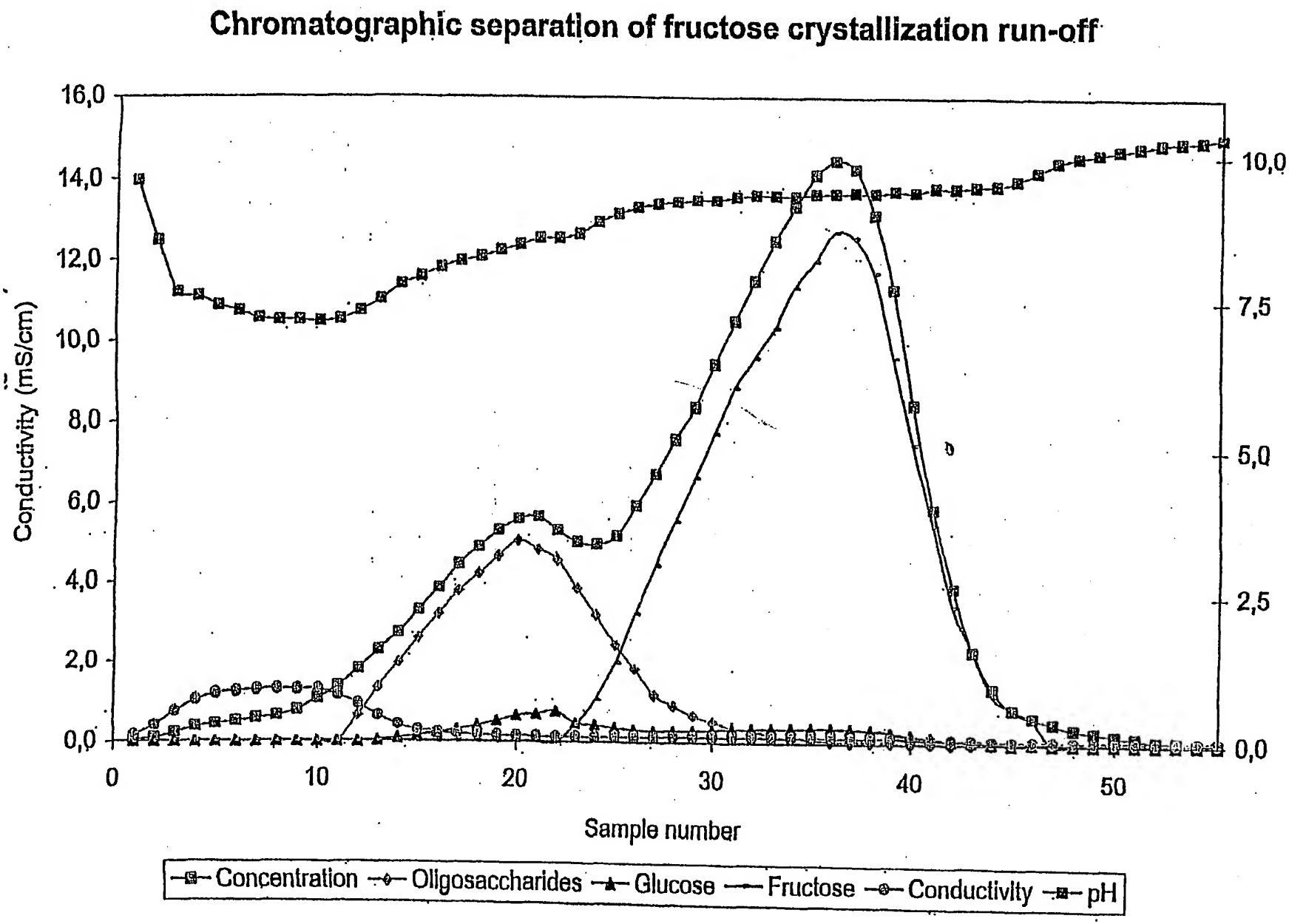
Chromatographic separation of fructose crystallization run-off
Conductivity (mS/cm)
pH
Sample number
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
10,0
7,5
5,0
2,5
0,0
0
10
20
30
40
50
Concentration
Oligosaccharides
Glucose
Fructose
Conductivity
pH

FIG. 7

INTERNATIONAL SEARCH REPORT

Inte. ional application No.
T/FI 01/00846

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C13J 1/06, C13K 13/00, C13D 3/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C13J, C13K, C13D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI DATA, PAJ, EPO-INTERNAL, CHEM.ABS.DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STN International, file CAPLUS, CAPLUS, accession no 127:47442, Oishi Kazuyuki et al: "Liquid column chromatography packed with two media for efficient separation of sugars", & Jpn. Kokai Tokkyo Koho JP 09127090,A2,19970516 Heisei, 5 pp. (Japanese) --	1-39
X	US 5998607 A (HEIKKILÄ ET AL), 7 December 1999 (07.12.99), see example 10,11 --	1-39
X	US 4904769 A (RAUENBUSCH), 27 February 1990 (27.02.90), page 1, line 38 - page 2, line 21 --	1-39

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2002	08-02-2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer ANNA SJÖLUND/BS Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

27/12/02

International application No.
PCT/FI 01/00846

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5998607 A	07/12/99	AU 3178997 A	14/01/98
		CA 2258866 A	31/12/97
		CZ 9804090 A	14/04/99
		EP 0914312 A	12/05/99
		FI 102962 B	00/00/00
		FI 962610 A	25/12/97
		JP 2000511199 T	29/08/00
		SK 175998 A	11/06/99
		WO 9749659 A	31/12/97
US 4904769 A	27/02/90	AT 71951 T	15/02/92
		BG 49497 A	15/11/91
		CA 1288768 A	10/09/91
		CN 1013866 B	11/09/91
		CN 86108259 A	29/07/87
		DE 3543999 A	19/06/87
		DE 3683611 A	05/03/92
		DK 164870 B,C	31/08/92
		DK 598686 A	14/06/87
		EP 0226121 A,B	24/06/87
		SE 0226121 T3	
		ES 2038591 T	01/08/93
		HU 43083 A	28/09/87
		HU 196219 B	28/10/88
		JP 2502551 B	29/05/96
		JP 2628853 B	09/07/97
		JP 8245683 A	24/09/96
		JP 62155288 A	10/07/87
		KR 9404065 B	11/05/94